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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/506,877	07/29/2005	Steven P. Gygi	56954 (70207)	3582

21874 7590 01/25/2008

EDWARDS ANGELL PALMER & DODGE LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
FOSTER, CHRISTINE E	
ART UNIT	**PAPER NUMBER**
1641	

MAIL DATE	DELIVERY MODE
01/25/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/506,877	GYGI ET AL.	
	Examiner	Art Unit	
	Christine Foster	1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 November 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-44* is/are pending in the application.

4a) Of the above claim(s) *30-44* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-29* is/are rejected.

7)☒ Claim(s) *1-3,5,7,8,12,13 and 29* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *9/3/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of Group I, claims 1-26 and 28-29 in the reply filed on 11/2/07 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

2. Since a search of the claims of Group II could also be performed without undue burden, **the restriction requirement between Groups I and II as set forth in the prior Office action is hereby withdrawn**.

3. Claims 30-44 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected inventions, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 11/2/07 as discussed above.

Accordingly, claims 1-29 are subject to examination below.

Priority

4. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

5. If applicant desires to claim the benefit of a prior-filed application under 35 U.S.C. 119(e), a specific reference to the prior-filed application in compliance with 37 CFR 1.78(a) must be included in the first sentence(s) of the specification following the title or in an

application data sheet. For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of the applications.

If the instant application is a utility or plant application filed under 35 U.S.C. 111(a) on or after November 29, 2000, the specific reference must be submitted during the pendency of the application and within the later of four months from the actual filing date of the application or sixteen months from the filing date of the prior application. If the application is a utility or plant application which entered the national stage from an international application filed on or after November 29, 2000, after compliance with 35 U.S.C. 371, the specific reference must be submitted during the pendency of the application and within the later of four months from the date on which the national stage commenced under 35 U.S.C. 371(b) or (f) or sixteen months from the filing date of the prior application. See 37 CFR 1.78(a)(2)(ii) and (a)(5)(ii). This time period is not extendable and a failure to submit the reference required by 35 U.S.C. 119(e) and/or 120, where applicable, within this time period is considered a waiver of any benefit of such prior application(s) under 35 U.S.C. 119(e), 120, 121 and 365(c). A benefit claim filed after the required time period may be accepted if it is accompanied by a grantable petition to accept an unintentionally delayed benefit claim under 35 U.S.C. 119(e), 120, 121 and 365(c). The petition must be accompanied by (1) the reference required by 35 U.S.C. 120 or 119(e) and 37 CFR 1.78(a)(2) or (a)(5) to the prior application (unless previously submitted), (2) a surcharge under 37 CFR 1.17(t), and (3) a statement that the entire delay between the date the claim was due under 37 CFR 1.78(a)(2) or (a)(5) and the date the claim was filed was unintentional. The Director may require additional information where there is a question whether the delay was

unintentional. The petition should be addressed to: Mail Stop Petition, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

If the reference to the prior application was previously submitted within the time period set forth in 37 CFR 1.78(a), but not in the first sentence(s) of the specification or an application data sheet (ADS) as required by 37 CFR 1.78(a) (e.g., if the reference was submitted in an oath or declaration or the application transmittal letter), and the information concerning the benefit claim was recognized by the Office as shown by its inclusion on the first filing receipt, the petition under 37 CFR 1.78(a) and the surcharge under 37 CFR 1.17(t) are not required. Applicant is still required to submit the reference in compliance with 37 CFR 1.78(a) by filing an amendment to the first sentence(s) of the specification or an ADS. See MPEP § 201.11.

Information Disclosure Statement

6. Applicant's Information Disclosure Statement filed 9/3/04 has been received and entered into the application. The references therein have been considered by the examiner as indicated on the attached form PTO-1449.

Specification

7. The specification is objected to for the following reasons:

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1 .821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1 .821 through 1 .825 for the reason(s) set forth below.

It appears that while Applicant has successfully submitted sequences in a computer readable form, the specification is not compliant with sequence rules. Specifically, **Figures 1, 5A-5C, 6, 9-10,** of the instant specification contains amino acid sequences that are not identified by SEQ ID numbers. The specification at **page 11** also refers to amino acid sequences not identified by SEQ ID numbers.

Applicant is required to review the instant application for compliance with the requirements of applications which contain sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821-1.825.

If the noted sequence(s) is in the sequence listing filed, Applicants must amend the specification to identify the sequence appropriately by SEQ ID NO. If the noted sequence(s) is not in the sequence listing as filed, Applicants must provide (1) a substitute copy of the sequence listing in both computer readable form (CRF) and paper copy, (2) an amendment directing its entry into the specification, (3) a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d), and (4) any amendment to the specification to identify the sequences appropriately by SEQ ID NO.

Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR 1.821(g).

Applicant's time to comply with the sequence rules is set forth on the attached Office Action Summary (Form PTOL-326). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a). In no case may an applicant extend the period for reply beyond the SIX MONTH statutory period. Direct the reply

to the undersigned.

Claim Objections

8. Claims 1-3, 5, 7-8, 12-13 and 29 are objected to because of the following informalities:

9. Claims 1-2 are objected to for the following reasons. Claim 1 recites the step of digesting ubiquitinated polypeptides to generate a plurality of test peptides, followed by the step of determining the presence of an isopeptide bond "in a test peptide". This reference to "a test peptide" is apparently referring to one of the plurality of test peptides mentioned previously in the claim. Similarly, claim 2 refers to "a test peptide" in line 6, which is apparently referring to one of the "plurality of test peptides" recited in line 4. However, this is not made clear since there is no recited connection between the "plurality of test peptides" and the "test peptide" later recited in these claims. Applicant is requested to clarify claim 1 in order to convey that the test peptide in which the presence of an isopeptide bond is determined is one of the plurality of test peptides generated by digestion in the preceding step. Similarly, Applicant is requested to clarify claim 2 in order reflect that the test peptide in which a mass difference is identified is one of the test peptides generated by digestion.

10. Claims 3 and 5 are objected to because it refers to "a test peptide", which presumably refers back to the plurality of test peptides mentioned in claim 1. However, the claims fail to recite any connection to the test peptide(s) recited in the independent claim. Therefore, the claims are confusing because is unclear what test peptide is being ionized. Applicant is requested to clarify how the dependent claims fit into the method of claim 1.

11. Claim 7 refers to "ubiquitinated polypeptides", which apparently refers to the ubiquitinated polypeptides recited in independent claim 1. It is suggested that the dependent claim refer to --the-- ubiquitinated polypeptides or similar language in order to make this clear.

12. Claim 8 recites "the ubiquitinated peptides", which should apparently read --the ubiquitinated **poly**peptides-- for consistency.

13. Claims 12-13 are objected to for the following reasons. Claim 12 recites the further step of detecting multiple ubiquitination sites in "a single polypeptide". This reference would appear to refer to one of the ubiquitinated polypeptides earlier recited in claim 1, but this is not made clear. Similarly, claim 13 refers to determining the relative abundance of ubiquitination in "a plurality of polypeptides". It is suggested that the claims be amended to clarify how the polypeptide(s) recited in dependent claims 12-13 correspond to the plurality of ubiquitinated polypeptides of claim 1.

14. Claim 29 contains a typographical error in referring to "claim f or 2", which should apparently read --claim 1 or 2'--.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

15. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

16. Claims 1-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not

described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The MPEP states that the purpose of the written description requirement is to ensure that the inventor had possession, as of the filing date of the application, of the specific subject matter later claimed. The MPEP lists factors that can be used to determine if sufficient evidence of possession has been furnished in the disclosure of the application. These include "level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention." MPEP 2163.

The nature of the invention relates to a mass spectrometric method for determining the site of ubiquitination on a polypeptide (i.e., the residue in the polypeptides to which ubiquitin is attached). In particular, the specification discloses that a sample containing ubiquitinated proteins can be digested with a protease such a trypsin (see especially page 32). Trypsin fragments the protein into test peptides, some of which will contain the lysine residue to which ubiquitin was attached as well as the ubiquitin remnant Gly-Gly, which has a mass of approximately 114 Daltons (ubiquitin, being also a protein, is also digested by trypsin to produce this Gly-Gly remnant). See page 8, last paragraph and page 32, penultimate paragraph. The Gly-Gly remnant produces a mass difference of 114 Daltons which can then be detected by mass spectrometry as indicative of the ubiquitination site.

Therefore, the specification describes methods of detecting the isopeptide bond formed between a protein lysine residue and the trypsin-generated ubiquitin remnant Gly-Gly, by mass spectrometrically detecting the 114 Da mass difference attributable to this remnant.

However, independent claim 1 broadly encompasses a genus of methods for determining ubiquitination sites by determining the presence of any type of isopeptide bond. The claims do not require that the isopeptide bond determined is one that is formed **between ubiquitin and the ubiquitinated polypeptide**. Since only those isopeptide bonds formed *with ubiquitin* would be indicative of the ubiquitination site, one skilled in the art would not envisage possession of methods of determining a ubiquitination site by determining an isopeptide bond corresponding to an intermolecular crosslink with a non-ubiquitin protein, for example. Furthermore, it is known that ubiquitin forms isopeptide bonds with *internal lysine residues* of target proteins. See for example Piotrowski et al. (*Journal of Biological Chemistry* Vol. 272 (1997), pages 23712-23721, of record) at page 23712, right column, the first paragraph. Therefore, the specification fails to convey evidence of possession of methods of determining isopeptide bonds not involving lysine residues.

Similarly, independent claim 2 broadly encompasses methods of detecting a genus of ubiquitin remnants, produced by trypsin or by other proteases, other than the disclosed ubiquitin remnant Gly-Gly. As discussed above, the specification only describes with any particularity methods of detecting the isopeptide bond formed between a protein lysine residue and the trypsin-generated ubiquitin remnant Gly-Gly, by mass spectrometrically detecting the 114 Da mass difference attributable to this remnant. This disclosure does not adequately support the claimed genus of "ubiquitin remnants".

The specification does suggest that other proteases besides trypsin could be used to fragment the ubiquitinated polypeptides (page 17, last paragraph). However, the specification does not disclose the structure of any other ubiquitin remnant other than Gly-Gly. It is not disclosed what ubiquitin remnants would be expected when other proteases besides trypsin would be used according to the claimed invention. Without knowledge of what ubiquitin remnants would be produced by other proteases, and what masses such remnants would have that could then be detected by mass spectrometry as a mass difference, one skilled in the art cannot envisage the structure of the isopeptide bonds to be determined by the claimed method. Similarly, with the exception of the 114 Da ubiquitin remnant Gly-Gly, the structure(s) of other ubiquitin remnants and the mass differences attributable to such remnants cannot be clearly envisaged by the skilled artisan. Applicant has only described the Gly-Gly ubiquitin remnant with any specificity.

As an illustrative example, Denis et al. ("Tryptic digestion of ubiquitin standards reveals an improved strategy for identifying ubiquitinated proteins by mass spectrometry" Proteomics. 2007 Mar;7(6):868-74) teach a mass spectrometric method for identifying ubiquitinated proteins involving trypsin digestion as in the instant specification. Denis et al. report that ubiquitination sites can be identified by ubiquitin remnants ("signature peptides") containing the 114.1 Da Gly-Gly tag, as in the instant specification (see the abstract). However, Denis et al. report that trypsin digestion also produced a LRGG ubiquitin remnant, which could also be detected by mass spectrometry as a 383.2 Da mass difference and used in the same way as the 114 Da Gly-Gly remnant to indicate the site of ubiquitination.

The recent findings of Denis et al. indicate that the detection of specific protease-derived ubiquitin remnants continues to be an area of active research.

Applicant's claims would encompass not only detection of isopeptide bonds involving the 114 Da Gly-Gly ubiquitin remnant, but also the 383.2 Da LRGG ubiquitin remnant of Denis et al., of which there is no description in the specification.

Similarly, Wang et al. (discussed further below) report a 270 Da Arg-Gly-Gly ubiquitin remnant remaining following digestion with trypsin.

One skilled in the art would not envisage possession of methods of determining ubiquitin sites by detecting isopeptide bonds involving the LRGG ubiquitin remnant (for example), given that the specification only describes detection of isopeptide bonds involving the trypsin-generated ubiquitin remnant Gly-Gly, via mass spectrometric detection of a 114 Da mass difference. The specification fails to convey evidence of possession of methods of detecting any isopeptide bond (i.e., involving any other ubiquitin remnant other than Gly-Gly), since neither the particular structure of the other ubiquitin remnants to be detected nor their expected masses are disclosed.

The findings of Denis et al. and Wang et al. also indicate unpredictability in the art, in that the same protease (trypsin) produced distinct ubiquitin remnants having different associated mass differences. One skilled in the art would not envisage methods involving detection of *any* ubiquitin remnant generated by digestion with *any* protease, since it is not disclosed what ubiquitin remnants and what mass changes would result as a result of digestion with other proteases besides trypsin. With the exception of the 114 Da Gly-Gly remnant, the detailed structure of the ubiquitin remnants to be detected cannot be envisaged.

Although claim 11 refers to Gly-Gly amino acid residues, the claim employs open transitional language "comprising", such that the ubiquitin remnant referred to could include additional non-disclosed amino acids at either end of this dipeptide sequence. However, Applicant has only disclosed methods of producing and analyzing the 114 Da ubiquitin remnant that consists of Gly-Gly, and therefore fails to convey evidence of possession of all ubiquitin remnants that include this sequence.

Similarly, although claim 27 recites that the mass difference is about 114 daltons, there is no requirement that the ubiquitin remnant be Gly-Gly. Applicant has not described any other structures that would produce a mass difference of 114 Da, and therefore fails to convey evidence of possession of the genus of ubiquitin remnants having this same mass.

Accordingly, it is deemed that the specification fails to provide adequate written description for the genus of the claims and does not reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the entire scope of the claimed invention.

17. Claims 7 and 14-18 also lack adequate written description for the following reasons. Claim 7 recites "binding partners which bind to a ubiquitin molecule". Claim 14 further specifies that the binding partners specifically bind to a "tag molecule" linked to ubiquitin.

In the instant case, the claims encompass a genus of binding partners that possess the functional characteristic of being able to bind to "a ubiquitin molecule" (and/or a tag molecule linked to ubiquitin). The claims are not adequately described by the specification because the

identification of the recited binding partners by reference to this desired functional characteristic alone is not sufficient.

The specification indicates that the binding partners may be antibodies that bind to ubiquitin (page 33, second paragraph).

The courts have stated that "as long as an applicant has disclosed a "fully characterized antigen," either by its structure, formula, chemical name, or physical properties, or by depositing the protein in a public depository, the applicant can then claim an antibody by its binding affinity to that described antigen." *Noelle v. Lederman*, 355 F.3d at 1349 (Fed. Cir. 2004, emphasis in the original). Although *Noelle* relates to antibodies *per se* and not to detecting methods using such antibodies, the holdings of those cases are also applicable to claims such as those at issue here. A disclosure that does not adequately describe a product itself logically cannot adequately describe a method of using that product.

Therefore, Applicant has adequately described *antibodies* capable of binding to ubiquitin *per se*, since this represents a fully characterized antigen.

However, claim 7 is not limited to binding partners that are antibodies. The specification does not disclose any shared partial structure or other distinctive characteristics possessed by the members of the genus. No other binding partners, other than antibodies, are disclosed with any particularity. The characteristics of the genus are not known, because there is no disclosure of what structure(s) would confer the necessary binding characteristics. There is no disclosed correlation between structure and function.

In addition, claim 7 recites binding partners which bind to "**a ubiquitin molecule**". Dependent claim 14 indicates that the binding partners may bind to a tag molecule attached to

ubiquitin. Therefore, the terminology "**a ubiquitin molecule**" would broadly encompass not only the ubiquitin protein *per se* but also various modifications of the ubiquitin protein: the addition of tags as in claim 14, as well as non-disclosed modification such as mutations, deletions, insertions, or other variants of ubiquitin. The specification does not adequately define what structural features commonly possessed by members of the genus of "ubiquitin molecules". One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus.

Therefore, although antibodies capable of binding to ubiquitin *per se* are adequately described, the specification does not provide adequate written description of antibodies capable of binding to all "ubiquitin molecules", since such molecules do not represent a fully characterized antigen. Similarly, although the specification discloses examples of affinity tags, there is no disclosed or recited common structure that would serve to distinguish the claimed genus of "tag molecules" as in claim 14. Therefore, the structure of the recited "tag molecule" cannot be clearly envisaged by the skilled artisan; and in turn, antibodies that bind to such molecules are not adequately described.

18. Claim 28 also lacks written description for the following reasons. The claim recites that "the site of ubiquitination is correlated with disease and detection of ubiquitination at the site is associated with risk of the disease". The specification suggests that the method can be used to diagnose disease (page 5) but does not disclose with any particularity what ubiqutination sites on what proteins are correlated with specific disease(s), and with which disease(s). Such general statements regarding diagnostic applications are insufficient absent a disclosure of what

condition can be diagnosed. One skilled in the art cannot envisage, based on the specification, what ubiquitination site(s) on which proteins might be correlated with what disease conditions, and which sites might be associated with risk of such disease conditions.

Scope of Enablement

19. Claims 1-27 and 29 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods for determining a site of ubiquitination by determining the presence of an isopeptide bond formed between a lysine residue and the trypsin-generated ubiquitin remnants Gly-Gly or Arg-Gly-Gly by detecting mass differences of about 114 Da or about 270 Da, respectively, due to the ubiquitin remnants, or for methods of detecting a mass difference due to a Glu-C-generated ubiquitin remnant of 1303.6 Da, does not reasonably provide enablement for methods of determining ubiquitination sites by detecting *any* type of isopeptide bond or by detecting any mass difference attributable to any remnant of ubiquitin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The nature of the invention relates to a mass spectrometric method for determining the site of ubiquitination on a polypeptide (i.e., the residue in the polypeptides to which ubiquitin is attached). In particular, the specification discloses that a sample containing ubiquitinated proteins can be digested with a protease such a trypsin (see especially page 32). This fragments the protein into test peptides, some of which will contain the amino acid residue to which

ubiquitin was attached. Ubiquitin, being also a protein, is also fragmented by the protease to leave a ubiquitin remnant on the amino acid residue.

More particularly, the specification discloses that digestion of ubiquitinated proteins with the protease trypsin will produce ubiquitin remnants having the sequence Gly-Gly, which has a mass of approximately 114 Daltons (page 8, last paragraph and page 32, penultimate paragraph). It is this mass difference of 114 Daltons which can then be detected by mass spectrometry as indicative of the ubiquitination site.

However, claim 1 broadly encompasses methods for determining ubiquitination sites by determining the presence of any type of isopeptide bond. The claims do not require that the isopeptide bond determined is one that is formed **between ubiquitin and the ubiquitinated polypeptide**. Since only those isopeptide bonds formed *with ubiquitin* would be indicative of the ubiquitination site, undue experimentation would be required in order to determine a ubiquitination site by detecting an isopeptide bond corresponding to an intermolecular crosslink with a non-ubiquitin protein, for example. Furthermore, it is known that ubiquitin forms isopeptide bonds with *internal lysine residues* of target proteins, as taught by Piotrowski et al. as discussed further above. Therefore, the claims directed to determination of any type of isopeptide bond are inconsistent with the knowledge in the prior art that ubiquitin only forms isopeptide bonds with lysine residues on target proteins.

Similarly, claim 2 broadly encompasses methods in which the mass difference of *any* ubiquitin remnant is used to indicate the ubiquitination site. However, the specification only provides guidance with respect to detection of the 114 Da mass difference due to the Gly-Gly remnant that remains following digestion with trypsin.

The specification does suggest that other proteases besides trypsin can be used to fragment the ubiquitinated polypeptides (page 17, last paragraph). However, the specification fails to provide guidance in determining ubiquitin sites except by detecting the 114 Da mass difference attributable to the trypsin-generated ubiquitin remnant Gly-Gly. There are no working examples in which ubiquitin sites were detected except by detecting isopeptide bonds involving the 114 Da Gly-Gly ubiquitin remnant. Since the claimed methods involve detection of mass differences due to ubiquitin, knowledge of the expected size(s) of the ubiquitin remnants is necessary in order to carry out the claimed invention.

Denis et al. (discussed above) report that trypsin digestion of ubiquitinated polypeptides can produce multiple different ubiquitin remnants, namely the 114 Da Gly-Gly remnant disclosed in the instant specification as well as a 383.2 Da LRGG ubiquitin remnant. Wang et al. (discussed below) identified a 270-Da mass difference due to the ubiquitin remnant Arg-Gly-Gly following trypsin digestion. Such findings indicate unpredictability in the art, in that the same protease (trypsin) can produce distinct ubiquitin remnants having different associated mass differences.

Despite such unpredictability, the claims would encompass detection of isopeptide bonds on test peptides produced by treatment with any protease. The claims also broadly encompass non-disclosed methods of mass spectrometric detection of any type of isopeptide bond, such as detection of isopeptide bonds involving the 383.2 Da LRGG ubiquitin remnant disclosed by Denis et al.

The prior art also fails to support such breadth. Wang et al., Marotti et al., and Laub et al. (discussed in further detail below) teach methods in which mass spectrometry methods were

used to identify ubiquitination sites based on ubiquitin remnants remaining on proteins (via isopeptide bond linkage) after protease digestion. Although the teachings indicate that it was known in the art at the time of the invention to identify ubiquitination sites by detecting a 270-Da Arg-Gly-Gly ubiquitin remnant (see Wang et al.), by detecting the 114 Da Gly-Gly ubiquitin remnant disclosed instantly and as also disclosed by Marotti et al., or by detecting a 1303.6 Da ubiquitin remnant generated by Glu-C digest, the prior art fails to teach how to detect any type of isopeptide bond or any ubiquitin remnant of any size or structure in order to detect ubiquitination sites.

With respect to claims 12-13, which recite that multiple ubiquitination sites in a single polypeptide are detected, it is noted that the claims do not recite any methods steps or limitations as to the sample type, experimental conditions, etc. that would enable this outcome to be achieved. The claims apparently refer to the *results* of the method, yet it is unclear how this could be known beforehand unless (for example) the starting sample was in some way defined. The prior art teaches that not all proteins have multiple ubiquitination sites. For example, Laub et al. (discussed further below) studied bovine and recombinant calmodulin, and found that these polypeptides are ubiquitinated at a single site. The specification fails to enable the full scope of the claims, which would encompass detection of multiple ubiquitination sites in *any* protein (such as calmodulin, which is not recognized to be multiply ubiquitinated), in *any* starting sample, etc.

In summary, due to the lack of guidance with regard to detection of ubiquitination sites other than by detecting the 114 Da Gly-Gly ubiquitin remnants produced by trypsin, the lack of working examples directed to same, the breadth of the claims, and the unpredictability in the art,

the specification fails to teach the skilled artisan how to carry out the claimed invention in its full scope without undue experimentation.

Enablement

20. Claim 28 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

It is noted that the insufficiency of disclosure issues discussed above in relation to claims 1-26 and 28-29 would also apply equally to claim 28.

In addition, claim 28 recites that "the site of ubiquitination is correlated with disease and detection of ubiquitination at the site is associated with risk of the disease". When given its broadest reasonable interpretation, the claim invokes methods in which risk of disease is diagnosed based on the presence of ubiquitination at a particular site.

The courts have stated that "tossing out the mere germ of an idea does not constitute enabling disclosure." Genentech, 108 F.3d at 1366 (quoting Brenner v. Manson, 383 U.S. 519, 536 (1966) (stating, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion")). "[R]easonable detail must be provided in order to enable members of the public to understand and carry out the invention." Id.

In the instant case, such reasonable detail is lacking. The specification suggests that the method can be used to diagnose disease (page 5) but does not disclose with any particularity

what ubiqutination sites on what proteins are correlated with specific disease(s), and with which disease(s).

However, it is not disclosed what particular ubiquitination sites on which proteins would be correlated with disease, and with which disease. Such general statements regarding diagnostic applications are insufficient absent a disclosure of what condition(s) can be diagnosed.

The specification also suggests that ubiquitination patterns of normal cells could be compared with those of diseased cells (page 5). This general invitation to conduct further research to identify differences in disease states and to identify what ubiquitination sites are associated with disease is insufficient when the unpredictable nature of biomarker discovery and validation is taken into account.

In this regard, Bast et al. ("Translational Crossroads for Biomarkers" *Clin Cancer Res* 2005; 11(17), 6103-6108) point to the "lengthy process" of assay development and validation and note that many markers that correlate with disease statistically may not prove to be useful clinically (p. 6105, right column). See also LaBaer et al. ("So, You Want to Look for Biomarkers" *Journal of Proteome Research* 2005; 4, 1053-1059), which teaches that crucial validation steps are needed to demonstrate that an identified biomarker is a reliable predictor, and also that the process of converting such a biomarker into a practical clinical test is even more daunting (p. 1053, see the paragraph bridging the left and right columns). Baker ("In Biomarkers We Trust?" *Nature Biotechnology* 2005; 23(3), 297-304) also speaks to the unpredictability involved in clinically applying biomarkers (see p. 298, the section "Walking on Thin Ice"):

> "Using a new biomarker is like walking across a frozen lake without knowing how thick the ice is," says Ole Vesterqvist..."You start walking, and you get comfortable. Then you break through."

Thus, the state of the art teaches the unpredictability associated with the clinical use of biomarkers, even after a biomarker has been correlated with a specific disease state. These references teach to the unpredictability associated with validating candidate biomarkers for clinical use, as well as to the large quantity of experimentation that would be necessary to do so.

The claim would broadly encompass diagnosis of risk of any disease. There are no working examples in which detection of any ubiquitination site was correlated with risk of any specific disease. Given that Applicant has not disclosed any particular ubiquitination sites that are correlated with risk of any specific disease condition, the teachings of the specification do not bear a reasonable correlation to the scope of the claim.

21. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

22. Claims 1-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

23. Claims 1, 3-26 and 28-29 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. See MPEP § 2172.01. The omitted structural cooperative relationships are: that the isopeptide bond detected in the method is one that is formed between a lysine residue that is part of the amino acid sequence of the ubiquitinated polypeptide(s) and a Gly-Gly proteolytic fragment of ubiquitin.

Claim 1 recites that the presence of an isopeptide bond in a test peptide formed by proteolysis of ubiquitinated polypeptides is indicative of a site of ubiquitination. However, the claims fail to recite that the isopeptide bond detected is in any related to a bond with *ubiquitin.*

Since only those isopeptide bonds formed at the site of ubiquitination would be indicative of the site of ubiquitination, it is an essential to the performance of the method that the isopeptide bond detected is one at the site of ubiquitination—i.e., the isopeptide bond formed between lysine residues on target proteins and the Gly-Gly remnant of ubiquitin that remains attached to lysine residues following proteolytic digestion with trypsin.

24. Claim 2 recites the limitation "the test peptide" in lines 7 and 10. There is insufficient antecedent basis for this limitation in the claim because the claim refers to "a plurality of test peptides" as well as to "a test peptide", and there is no requirement that these be the same test peptides. Therefore, there is ambiguity as to which test peptide is being invoked.

25. Claim 2 recites a mass difference that "corresponds to" the mass of the ubiquitin remnant. The term "corresponds to" renders the claim is indefinite because it is unclear in what way the mass difference "corresponds" to the mass of the ubiquitin remnant. It is unclear what "corresponds to" would encompass, absent a specific or limiting definition for this term in the context in which it is currently being used. Corresponds to does not indicate what type of correspondence is required or what level of correspondence.

26. Claim 5 recites the limitation "the test peptide" in line 3. There is insufficient antecedent basis for this limitation in the claim because claim 1 refers to "a plurality of test peptides" and to "a test peptide", while claim 5 also earlier recites "a test peptide". Therefore, there is ambiguity as to which test peptide is being invoked.

27. Claim 8 recites the limitation "the step of isolating" in line 1. There is insufficient antecedent basis for this limitation in the claim.

28. Claim 8 recites the limitation "the ubiquitinated peptides" in line 1. There is insufficient antecedent basis for this limitation in the claim since claim 1 refers to "ubiquitinated polypeptides" but not to "ubiquitinated peptides".

29. Claim 11 recites the limitation "the ubiquitin remnant" in line 1. There is insufficient antecedent basis for this limitation in the claim.

30. Claims 12-13 are rejected as being vague and indefinite for the following reasons. Claim 12 recites a method that further comprises "detecting multiple ubiquitination sites in a single polypeptide". This conveys a further step of detecting, i.e. identifying, multiple ubiquitination sites in a polypeptide. However, not all polypeptides would have multiple ubiquitination sites. Therefore, the claim is indefinite because it is apparently referring to the *results* of the method, which would not yet be known. Independent claim 1 invokes a method of identifying ubiquitination sites. It is unclear how the methods of claims 12-13 would be carried out on the *unknown* sample invoked by claim 1, i.e. a sample in which the ubiquitin sites have not yet been identified. Detection of multiple sites of ubiquitination would apparently require it to be known beforehand that the ubiquitinated proteins provided are multiply ubiquitinated rather than ubiquitinated on a single site. Clarification is needed as to how detection of multiple sites would be accomplished--is a particular protein sample being provided, for example?

Claim Rejections - 35 USC § 102

31. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

32. Claims 1-6, 8-11 and 29 are rejected under 35 U.S.C. 102(a) as being anticipated by Wang et al. ("Antibacterial peptides in stimulated human granulocytes Characterization of ubiquitinated histone H1A" Eur J Biochem. 2002 Jan;269(2):512-8).

Wang et al. teach providing a ubiquitinated polypeptide fragment of histone H1A corresponding to Lys152-Lys222 of histone H1A, digesting the polypeptide with trypsin to generate resulting test peptides, and identifying the site of ubiquitination by determining the presence of an isopeptide bond between the ε-amino side chain of Lys222 of H1A and a ubiquitin remnant corresponding to the three C-terminal residues of ubiquitin (Arg-Gly-Gly) by mass spectrometry (ES MS and MS/MS) See in particular the abstract; page 515 to page 516, first paragraph; Figure 4 and legend; and page 517, left column, first full paragraph; as well as pages 513-514, the sections "Extraction and purification of proteins..."; "Protein analysis"; and "Mass spectrometry"; pages 514-515, the section "Purification and identification..." and especially the paragraph bridging pages 514-515; and page 517, left column). Wang et al. identified a 270-Da mass difference due to the tripeptide ubiquitin remnant (page 514, right column, last paragraph to page 515; Table 2; Scheme 2; and page 517, left column, the first full

paragraph. It is noted that the sample of ubiquitinated H1A fragment subjected to trypsin digestion constitutes a "plurality" of ubiquitinated polypeptides since it contained multiple molecules of this polypeptide, as indicated by the fact that trypsin digested some of the molecules but not all (page 516, first paragraph).

With respect to claims 3-4 and 6, the mass spectrometric procedures of Wang et al. involved fragmentation of ions by ES (electrospray) MS (page 516, right column).

With respect to claim 5, Wang et al. teach comparing the masses of test peptides as determined by mass spectrometry to the predicted sequences expected based on the primary structure of histone H1a (page 514, right column; page 516, right column). The reference also teaches conducting a database search of the sequence Arg-Gly-Gly (see the paragraph bridging pages 514-515), which would also read on the instant claim.

With respect to claims 8-10, Wang et al. teach obtaining the ubiquitinated H1A fragments by RP-HPLC (see page 514, the section "Purifcation and identification of ubiquitinated histone H1A (uH1A) fragments"; and Figure 1B).

With respect to claim 11, Wang et al. detected the ubiquitin remnant Arg-Gly-Gly (see for example scheme 2), which comprises Gly-Gly.

With respect to claim 29, Wang et al. also determined the presence or absence of a cleavage site, i.e. modification (page 516, right column).

33. Claims 1-6, 8-11, 27 and 29 are rejected under 35 U.S.C. 102(a) as being anticipated by Marotti et al. ("Direct identification of a G protein ubiquitination site by mass spectrometry" Biochemistry Vol. 41(16):5067-74, Published on Web 2/27/02).

Marotti et al. teach a method for identifying a ubiquitination site on the G protein subunit Ga1, comprising obtaining a plurality of ubiquitinated Gpa1 polypeptides from a yeast expression system, and digesting the polypeptides (provided as gel slices) with the protease trypsin. See the abstract; page 5068, left column to page 5069, right column; page 5072, left column, the first two paragraphs; and page 5073, right column, last two lines to page 5074, first paragraph; and especially at pages 5068-5069, the section "Mass Spectrometry" and Figure 1. The resulting peptides were then analyzed by LC-MS/MS (page 5069, left column). Mass spectrometry identified a mass difference in a tryptic fragment that corresponded to Gpa1 residues 168-178 (i.e., reference peptide) plus a 115 Da modification indicating attachment of the ubiquitin remnant Gly-Gly in isopeptide linkage with Lys 165 (page 5069, right column, last paragraph and Figure 1 and legend).

With respect to claims 3 and 6, Marotti et al. teach electrospray ionization methods (page 5069). With respect to claim 4, the ions were fragmented by collision-induced dissociation (CID; ibid).

With respect to claims 8-10, Marotti et al. teach reverse-phase liquid chromatography (page 5069, left column).

With respect to claim 11, the ubiquitin remnant Gly-Gly was detected as discussed above (page 5069, right column, last paragraph).

With respect to claim 27, the Gly-Gly remnant with a mass difference of 115 Da would read on the instantly claimed difference of "about" 114 Da.

With respect to claim 29, Marotti et al. also determined sites of modification (cleavage) by trypsin (page 5069, right column, last paragraph and Figure 1).

34. Claims 1-6, 8-11, and 29 are rejected under 35 U.S.C. 102(b) as being anticipated by Laub et al. ("Modulation of calmodulin function by ubiquitin-calmodulin ligase and identification of the responsible ubiquitylation site in vertebrate calmodulin" (1998) Eur. J. Biochem. 255: 422–431).

Laub et al. teach determination of the ubiquitination site of calmodulin by mass spectrometry, in particular by providing a plurality of ubiquityl-calmodulin conjugates (bovine testis or "BT-calmodulin" as well as recombinant *Xenopus laevis* or "r*Xl*-calmodulin"), digesting the conjugates with CNBr as well as with the protease Glu-C; and performing CID-mass spectrometry. The mass spectrometric analysis observed five fragments diagnostic for ubiquitination on Lys21, as indicated by a mass difference of 1303.6 Da due to a ubiquitin fragment, while fragments that would correspond to the expected mass due to ubiquitination on Lys30 were not observed (pages 427-428; Figures 7-8; and Table 2). The detection of ubiquitination on Lys21 amounts to determination of the presence of an isopeptide bond, as ubiquitin is attached to calmodulin via an isopeptide bond as depicted in Figure 7, bottom panel.

See in particular the abstract; the paragraph bridging pages 422-423; page 423-424, "Materials and Methods"; page 425-429, the section "Structural analysis of ubiquityl-calmodulins"; and especially at page 423, the section "Fragmentation of Conjugates" and the right column, last paragraph to page 424, left column; at page 426, last paragraph to page 429, first paragraph.

With respect to claims 3-4 and 6, Laub et al. teach nanospray electrospray mass spectrometry and fragmentation of ions by collision-induced dissociation (CID; see page 424, left column; and Figures 6 and 8).

With respect to claims 8-10, Laub et al. teach HPLC and reverse-phase separation of the protease-digested peptides (page 423, last paragraph; page 426, last paragraph).

With respect to claim 11, the 13-3.6 Da ubiquitin remnant comprises Gly-Gly (GG) as seen in Figure 7, bottom panel.

With respect to claim 29, the mass spectrometry analysis also determined the site of protease modification (cleavage) as seen in Figure 8, for example.

Claim Rejections - 35 USC § 103

35. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

36. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

37. Claims 7, 19-22, and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. (US 2005/0287608 A1).

Laub et al. and Marotti et al. are as discussed above, which teach methods for identifying the ubiquitination site on calmodulin-ubiquitin conjugates by mass spectrometry. However, the references fail to specifically teach identifying ubiquitination sites for a plurality of polypeptides *in a first cell* as in claim 19. The references also fail to specifically teach that the ubiquitinated calmodulin conjugates are obtained by contacting cellular polypeptides with binding partners that bind to a ubiquitin molecule, as in claim 7.

Madura et al. teach methods for establishing protein profiles, in which a plurality of ubiquitinated proteins are obtained by contacting a biological sample with a ubiquitin-binding protein, such as an anti-ubiquitin antibody (see in particular the abstract and paragraphs 8-11, 16-20). This initial purification step permits selective purification of ubiquitinated proteins, which represent a set of important cellular proteins (paragraphs 59 and 61). The isolated ubiquitinated proteins can then be subjected to limited proteolytic digestion (e.g. with trypsin) followed by mass spectrometry, which permits identification of the ubiquitinated cellular proteins (paragraphs 27-28, 35, 65, 67, 97). This protein profiling method can be used as part of a "proteomics" approach to determine the presence of aberrant or pathological conditions in a cell using control and pathological samples containing cells, such as blood, body fluids, etc. (paragraphs 3, 6-10, 29, 33, 60). For example, a protein profile can be used to examine the entire pool of ubiquitinated proteins in order to survey the levels of well-characterized targets that have been previously associated with disease [0032].

Therefore, it would have been obvious to one of ordinary skill in the art to extend the mass spectrometric methods of identifying ubiquitination sites of Laub et al. or Marotti et al., which focused on a single protein or class of proteins, to the study of the entire pool of ubiquitinated proteins as taught by Madura et al. In particular, it would have been obvious to provide a sample of cells (such as blood) as taught by Madura et al. and to isolate the ubiquitinated proteins therein using a ubiquitin-binding protein, followed by digestion of the isolated ubiquitinated proteins with trypsin and mass spectrometric analysis to identify sites of ubiquitination according to the methods of Laub et al. or Marotti et al.

Motivation to combine the reference teachings in this manner comes from the teachings of Madura et al. that the set of ubiquitinated proteins was recognized to be important, and further that this set of proteins can be studied by selectively purifying ubiquitinated proteins, allowing protein profiles to be generated. In addition, Madura et al. taught that the study of the protein complement of an organism ("proteomics") has emerged as an important approach for identifying drug targets and new drugs (paragraph 3). Therefore, the skilled artisan would be motivated to determine the ubiquitination sites of other proteins using the known techniques of Laub et al. or Marotti et al., and to study the entire pool of ubiquitinated proteins in a "proteomics" approach by incorporating the preliminary selective isolation step taught by Madura et al.

Additional motivation comes from the teachings of Madura et al. that obtaining a profile of all the ubiquitinated proteins in a cell can be used to determine the presence of aberrant or pathological conditions in a cell, as well as to survey the levels of targets previously associated with disease, etc.

One would have had a reasonable expectation of success because Madura et al. indicate that ubiquitinated proteins isolated from cells in this manner can be analyzed by mass spectrometry, which is the analysis method used by Laub et al. and Marotti et al. In addition, Marotti et al. clearly teach that their approach should be broadly applicable to the identification and characterization of other ubiquitinated proteins (page 5074).

With respect to claims 20-22, Madura et al. teach comparing a ubiquitinated protein profile to that typical of the presence of an aberrant condition or relative to a control sample from a healthy control, in order to indicate the presence of the aberrant condition (paragraphs 8, 16-24, 89-91). Therefore, it would have been further obvious to perform such a comparison step for the purposes of diagnosing aberrant or pathological conditions.

With respect to claim 25, Madura et al. teach drug screening methods in which the effect of a test compound is determined by analyzing a set of ubiquitinated proteins from a biological sample (including the cells therein) are exposed to a test compound (paragraphs 23-24 and 63). Therefore, it would have been further obvious to one of ordinary skill in the art to contact cell samples with a test drug compound and to compare the resulting ubiquitinated protein profile with that of a known healthy or disease profile, in order to assess potential therapeutic effects of the drug.

38. Claims 7 and 19-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Aebersold et al. (US 7,183,116 B2).

Laub et al. and Marotti et al. are as discussed above, which teach methods for identifying the ubiquitination site on calmodulin-ubiquitin conjugates by mass spectrometry. However, the

references fail to specifically teach identifying ubiquitination sites for a plurality of polypeptides *in a first cell* as in claim 19. The Laub et al. reference fails to specifically teach that the ubiquitinated calmodulin conjugates are obtained by contacting cellular polypeptides with binding partners that bind to a ubiquitin molecule, as in claim 7.

Aebersold et al. teach mass spectrometry-based methods for proteome analysis. The reference teaches that the classical biochemical approach to study biological processes has been based on sequential purification to homogeneity, assay, functional analysis, etc. (column 1, lines 10-30). By contrast, quantitative proteomics is the systematic analysis of all proteins expressed by a cell or tissue (column 2, lines 1-39). The methods of Aebersold et al. can be used for selective isolation of molecules from a sample, allowing quantitative analysis of complex mixtures of analytes (column 4, lines 19-62).

In addition to identification and quantification of proteins, other properties such as protein phosphorylation and other post translational modifications can be analyzed (column 4, lines 39-44; column 14, line 55 to column 15, line 33). Post translation modifications that may be analyzed include ubiquitination, in methods involving selective isolation of ubiquitinated proteins using an antibody having specific binding activity for ubiquitinated polypeptides (column 5, line 2; column 15, line 20; column 17, lines 6-16). The mass spectrometry analysis can be used to analyze complex biological specimens such as blood, urine, or other body fluids (column 16, line 31 to column 17, line 42).

Therefore, it would have been obvious to one of ordinary skill in the art to extend the teachings of Laub et al. and/or Marotti et al., in which proteins were sequentially studied and analyzed, to the systematic proteome analysis of all ubiquitinated proteins in a cell or tissue as

taught by Aebersold et al. In particular, it would have been obvious to selectively isolate ubiquitinated proteins from a complex mixture such as blood using an antibody having specific binding activity for ubiquitinated polypeptides as taught by Aebersold et al., followed by mass spectrometry analysis to determine the site(s) of ubiquitination according to the methods of Laub et al. or Marotti et al.

One would be motivated to do this in order to systematically analyze all ubiquitinated proteins expressed by a cell or tissue, as opposed to the classical biochemical approach (exemplified by Laub et al. and Marotti et al.) involving sequential purification and study of one protein or limited class of proteins at a time.

One would have had a reasonable expectation of success because Aebersold et al. indicate that ubiquitinated proteins that have been selectively isolated as discussed above may be subjected to mass spectrometry analysis, which is the method employed by Laub et al. and Marotti et al.

With respect to claims 20-22, Aebersold et al. teach comparative analysis of the molecular compositions of cells in different states (such as different pathological states) can be used to identify the molecular mechanisms that control transition between states (column 2). Therefore, it would have been further obvious to one of ordinary skill in the art to compare ubiquitination sites identified by the method of Laub et al. or Marotti et al. and Aebersold et al. in normal vs. pathological states in order to identify the underlying molecular mechanisms of a particular pathology.

39. Claims 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. as applied to claim 7 above, and further in view of Ling et al. ("Histidine-Tagged Ubiquitin Substitutes for Wild-Type Ubiquitin in Saccharomyces cerevisiae and Facilitates Isolation and Identification of in Vivo Substrates of the Ubiquitin Pathway" J Analytical Biochemistry 282, 54–64 (2000)), or in the alternative as being unpatentable over Laub et al. or Marotti et al. in view of Aebersold et al. as applied to claim 7 above, and further in view of Ling et al:

Laub et al., Marotti et al., Madura et al., and Aebersold et al. are as discussed above, which fail to specifically teach isolation of ubiquitinated polypeptides via binding partners that specifically bind to a tag molecule linked to ubiquitin. Marotti et al. does teach ubiquitinated polypeptides isolated from cells expressing myc-tagged ubiquitin (page 5068, left column, "Strains and Plasmids"); however, the tag is not used in the instance as a means of isolating the ubiquitinated proteins.

Ling et al. teaches a general method for purifying substrates of the ubiquitin pathway (i.e., ubiquitinated proteins) using yeast strains expressing histidine-tagged ubiquitin (the abstract and page 54, last paragraph to page 55, second paragraph). The ubiquitinated proteins can be subsequently isolated by metal chelation chromatography on Ni-NTA agarose, which specifically binds to the histidine tag (pages 56-57, "Cell extracts and metal ion chelation chromatography" and page 58, "Enrichment of in vivo HisUb-protein conjugates by metal ion chelation chromatography"). This purification approach is desirable since it allows for isolation of all ubiquitinated proteins (see the paragraph bridging pages 54-55).

Ling et al. also teach that while other tagged forms of ubiquitin have been used, such as myc-tagged ubiquitin, histidine-tagged ubiquitin is distinguished from such other tags in that it acts as a purification aid, offering affinity binding on a renewable matrix (page 62, left column, the second full paragraph).

Therefore, it would have been further obvious to one of ordinary skill in the art to selectively isolate the ubiquitinated proteins in the methods of Laub et al. or Marotti et al. and Madura et al. or Aebersold et al. discussed above according to the methods of Ling et al. In particular, it would have been obvious to employ a yeast cell expressing histidine-tagged ubiquitin and to isolate the ubiquitinated polypeptides using metal chelate affinity chromatography using binding partners (Ni-NTA) specific for the histidine tag, in order to selectively isolate the ubiquitinated proteins. This use of histidine-tagged ubiquitin as a purification aid is particularly pertinent to the methods of Laub et al. or Marotti et al. and Madura et al. or Aebersold et al., which involve the selective isolation of ubiquitinated proteins prior to mass spectrometric analysis as discussed above.

40. Claims 17-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. and Ling et al. as applied to claim 16 above, and further in view of Figueiredo-Pereira et al. ("Accumulation of ubiquitinated proteins in mouse neuronal cells induced by oxidative stress" Mol Biol Rep. 1997 Mar;24(1-2):35-8); or in the alternative as being unpatentable over Laub et al. or Marotti et al. in view of Aebersold et al. and Ling et al. as applied to claim 16 above, and further in view of Figueiredo-Pereira et al.

The references are as discussed above. Ling et al. teaches expression of ubiquitinated polypeptides in yeast cells that express histidine-tagged ubiquitin, but fails to specifically teach expression in mammalian or mouse cells.

Figueiredo-Pereira et al. analyzed the level of ubiquitinated proteins in mouse neuronal cells in response to oxidative stress, and found that the heavy metal cadmium induced the accumulation of ubiquitinated proteins in a dose- and time-dependent manner (the abstract and page 38).

Therefore, it would have been further obvious to one of ordinary skill in the art to apply the methods of Laub et al. or Marotti et al. and Aebersold et al. or Madura et al. and Ling et al. to the study of ubiquitinated proteins in mouse neuronal cells expressing histidine-tagged ubiquitin in order to study the effects of oxidative stress on ubiquitination. When taken together with successful use of mouse cells as a model system for determining the involvement of ubiquitination in oxidative stress, it would have been obvious to employ this known model system in order to analyze proteins ubiquitinated in response to oxidative stress.

41. Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. as applied to claim 22 above, and further in view of Figueiredo-Pereira et al., or in the alternative as being unpatentable over Laub et al. or Marotti et al. in view of Aebersold et al. as applied to claim 22 above, and further in view of Figueiredo-Pereira et al.

The references are as discussed above. Madura et al. teaches comparison of aberrant cells and normal cells, for example to determine the presence of a disease condition. Similarly,

Aebersold et al. teaches assessment of differences between cells in different states, e.g. pathological states (column 2). However, the references fail to specifically teach neurodegenerative disease.

Figueiredo-Pereira et al. teach that ubiquitin protein conjugates are commonly detected in neuronal brain inclusions of patients with neurodegenerative disorders (the abstract).

Therefore, it would have been obvious to one of ordinary skill in the art to generate a protein profile for neurodegenerative disorders in the methods of Laub et al. or Marotti et al. and Madura et al. in order to allow for a signature ubiquitinated protein profile for such disorders to be generated, which would be useful as a diagnostic tool or in screening for new therapeutic targets. Similarly, it would have been obvious to one of ordinary skill in the art to assess differences in cells with respect to their state neurodegenerative disorder (i.e., presence or absence of disorder) in the methods of Laub et al. or Marotti et al. and Aebersold et al. in order to identify the molecular mechanisms that control the transition to neurogenerative disease. One would have had a reasonable expectation of success because the teachings of Figueiredo-Pereira et al. establish that ubiquitination protein profiles were in fact known to be altered in neurodegenerative disorders.

42. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. as applied to claim 20 above, and further in view of Yang et al. ("Ubiquitin Protein Ligase Activity of IAPs and Their Degradation in Proteasomes in Response to Apoptotic Stimuli" Science 5 May 2000:Vol. 288. no. 5467, pp. 874 - 877)

The references are as discussed above. Madura et al. teaches comparison of aberrant cells and normal cells, for example to determine the presence of a disease condition, but fails to specifically teach comparison of cells that differ with respect to the expression of a recombinant DNA molecule.

Yang et al. teach that protein ubiquitination involves the sequential action of ubiquitin activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin protein ligase (E3) (see abstract page 874, right column to page 875, left column). Yang et al. took advantage of the fact that these enzymes, necessary for protein ubiquitination, are not expressed in prokaryotic cells in order to study protein ubiquitination. In particular, Yang et al. used bacterial cells expressing recombinant forms of the E1 and E2 as well as the IAP protein, which was found in their studies to possess endogenous ubiquitin ligase (E3) activity (page 875, left column). This model system allowed Yang et al. to determine that the IAP protein is capable of mediating its own ubiquitination and degradation in vivo, possessing endogenous ubiquitin ligase activity (page 875, left column to page 876, left column, first paragraph). In particular, Yang et al. compared protein ubiquitination patterns in cells that expressed recombinant IAP with those that did not express this molecule, and found that in the presence of IAP, a large increase in the number of ubiquitinated protein species was observed.

Yang et al. further teach that it remains to be determined whether IAP is capable of ubiquitinating other molecules and if so, what molecules (page 876, right column).

Therefore, it would have been obvious to one of ordinary skill in the art to employ the methods of Laub et al. or Marotti et al. and Madura et al. in order to study protein ubiquitination in bacterial cells using the model system of Yang et al. In particular, it would have been obvious

to compare protein ubiquitination sites in cells expressing the recombinant IAP protein with those that do not, as taught by Yang et al., in order to identify whether IAP is capable of ubiquitinating other molecules as suggested by Yang et al.

43. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over over Laub et al. or Marotti et al. in view of Aebersold et al. as applied to claim 20 above, and further in view of Yang et al.

The references are as discussed above. Aebersold et al. teaches assessment of differences between cells in different states, e.g. pathological states (column 2), but fails to specifically teach comparison of cells that differ with respect to the expression of a recombinant DNA molecule as in claim 24 or with respect to contact with a compound as in claim 25.

The teachings of Yang et al. are discussed immediately above. It is noted with respect to claim 25 that the model system taught by Yang et al. involved contacting the cells with recombinant IAP as taught on page 875, left column first paragraph.

Therefore, it would have been obvious to one of ordinary skill in the art to employ the methods of Laub et al. or Marotti et al. and Aebersold et al. order to study protein ubiquitination in bacterial cells using the model system of Yang et al. In particular, it would have been obvious to compare protein ubiquitination sites in cells expressing the recombinant IAP protein with those that do not, as taught by Yang et al., in order to identify whether IAP is capable of ubiquitinating other molecules as suggested by Yang et al.

44. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Laub et al. or Marotti et al. in view of Madura et al. as applied to claim 19 above, and further in view of Anderson et al. (US 2002/0087273 A1), or in the alternative as being unpatentable over Laub et al. or Marotti et al. in view of Aebersold et al. as applied to claim 19 above, and further in view of Anderson et al.

The references are as discussed above, which fail to specifically teach generating a database comprising data files as claimed.

Anderson et al. teach a computerized database is created for storing data relating to proteomic technology and for allowing user access to this data [0138]. The proteomics database can be used to compare samples from a treated patient with a protein index corresponding to normal samples to determine effectiveness of a therapy or biological effect of a candidate therapy [0251] and can also be used as a source of revenue [0252].

Therefore, it would have been obvious to one of ordinary skill in the art to compile the proteomics data obtained by the methods of Laub et al. or Marotti et al. and Madura et al. and Aebersold et al. into a computerized database as taught by Anderson et al. One would be motivated to this in order to store the data, to allow others to access the data, and/or as a source of revenue, for example.

Conclusion

45. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

46. Peng et al. ("A proteomics approach to understanding protein ubiquitination" Nature biotechnology, (2003 Aug) Vol. 21, No. 8, pp. 921-6) is cited as Applicant's postfiling work relevant to the claimed invention.

47. WO 03/102172 A1 (Anderson et al., not prior art on the currently presented claims with a priority date of 5/31/02) is cited as being relevant to the claimed invention for its teaching of identifying ubiquitinated proteins by mass spectrometry analysis of tryptic digests.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Christine Foster
Patent Examiner
Art Unit 1641

LONG V. LE 01/22/08
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600